TRICHOMONAS

Lack of evidence for the involvement of rectal and oral trichomonads in the aetiology of vaginal trichomoniasis in Ghana

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Objective: To investigate the possible involvement of human trichomonads (*Pentatrichomonas hominis* and *Trichomonas tenax*) other than *Trichomonas vaginalis* in the aetiology of vaginal trichomoniasis.
Methods: Vaginal swabs taken from women attending antenatal clinics were tested for *Trichomonas vaginalis* by traditional assays (wet-mount microscopy and InPouch culture) and nucleic acid amplification (polymerase chain reaction). These swabs were also tested for the presence of *P hominis* and *T tenax* by nucleic acid amplification. Oral and rectal swabs from these women were tested for *T tenax* and *P hominis* respectively. Data on sociodemographic characteristics, sexual and anogenital hygiene practices likely to seed *P hominis* and *T tenax* into the vagina were collected by a questionnaire.
Results: 93% (161) of the 173 samples in which *T vaginalis* was detected by wet preparation or culture was evaluable by PCR. Of this, *T vaginalis* was detected in 94% (152) by *T vaginalis*-specific PCR. Neither *P hominis* nor *T tenax* was detected in any of the vaginal swab samples. These included nine samples for which *T vaginalis* had been detected by wet preparation or culture, but were negative by *T vaginalis* nucleic acid amplification. *P hominis* and *T tenax* were not detected in any of the rectal and oral swabs, respectively.
Conclusion: In this group of women, there was no evidence for the involvement of trichomonads other than *T vaginalis* in the aetiology of vaginal trichomoniasis.

The causative agent of vaginal trichomoniasis is the flagellated protozoon parasite *Trichomonas vaginalis*. Evidence for this has come from epidemiological, laboratory and clinical studies conducted in the 1930s and 1940s.[1–4] The main diagnostic criterion used was the morphologically based wet preparation direct microscopy (wet prep), reported by Donné[5] in 1836. Two other trichomonads (*Pentatrichomonas hominis* and *Trichomonas tenax*) are morphologically identical with *T vaginalis* at microscopy, and some investigators have expressed caution at delineating all trichomonads found in the vagina as *T vaginalis*. *P hominis* is a commensal in the gut and *T tenax* is a commensal in the oral cavity. Walton and Bacharach[6] and Hersh[7] reporting on this diagnostic dilemma commented that morphological differentiation between the three trichomonads was difficult and that earlier investigators gave no reliable differentiation between them. This notwithstanding, Honigberg[8] and Wenrich[9] stated that these three species of trichomonads have distinct habitats and will not survive outside them.

Recent epidemiological and clinical studies have suggested the possible involvement of human trichomonads other than *T vaginalis* in the aetiology of vaginal trichomoniasis. Buvé *et al*[10] reported finding *T vaginalis* by microscopy in 40% of adolescent girls in Zambia, many of whom denied ever having sexual intercourse. The plausibility of finding *P hominis* in the vagina has been highlighted by a recent report detecting two cases in vaginal swabs using nucleic acid amplification techniques (NAAT).[11] Lurie and Glezerman[12] postulated that short anovaginal distances could influence conditions that promote microbial colonisation of the vagina. This was after they had isolated considerably more gut-related microbes (including *Trichomonad* sp—they did not mention which species) from the vagina of women with recurrent vaginitis than from controls.

In this study, we look at the possible involvement of *P hominis* and *T tenax* in the aetiology of vaginal trichomoniasis. Traditional trichomonad diagnostic tests were supplemented by NAAT that make trichomonad species differentiation possible.

The involvement of trichomonads other than *T vaginalis* in the aetiology of vaginal trichomoniasis could have implications for the control of vaginal trichomoniasis, as *P hominis* and *T tenax* have their reservoirs outside the urogenital tract.

METHODS

Details of the study population have been published previously.[13] Briefly, pregnant women attending antenatal care at four public hospitals in the Kumasi metropolis of Ghana between October 2002 and August 2003 participated in the study. Women consenting to participate in the study collected self-administered vaginal swabs that were tested for *T vaginalis* infection using a *T vaginalis* latex agglutination test (Kalon Biological, UK) All those testing positive and the next two consecutive patients testing negative were enrolled into the study. Vaginal examinations were carried out on these enrolled women, during which a nurse obtained three vaginal swabs from the posterior fornix after passage of a speculum. The swabs were tested for *T vaginalis* infection by wet preparation microscopy and culture. Women testing positive by either of these tests were enrolled as study cases, and those testing negative were controls. The participants answered a questionnaire seeking information on sociodemographic characteristics, sexual practices (including oral and rectal intercourse) and anogenital hygiene. Oral and rectal swabs were also collected by the nurses. Univariate analysis of associations between *T vaginalis* infection as determined by *T vaginalis* traditional testing (wet-mount microscopy and culture) and factors likely to transmit rectal and oral trichomonads into the vagina were

Abbreviations: LSHTM, London School Of Hygiene and Tropical Medicine; NAAT, nucleic acid amplification tehniques; PCR, polymerase chain reaction

determined by odds ratios and χ^2 and Fisher's exact tests. These factors included oral sex, rectal intercourse followed by vaginal penetration, poor anogenital hygiene with faecal staining of the perineum and practices of anogenital hygiene, including douching, and the direction of cleaning after defecation, postulating that cleaning forwards from the anus towards the vagina could introduce rectal trichomonads into the vagina.

The first vaginal swab was tested for *T vaginalis* by wet-mount microscopy, the second by culture with InPouch Tv (Biomed Diagnostics, San Jose, California, USA) and the third by polymerase chain reaction (PCR) assay for *T vaginalis*.

For the direct microscopy, the vaginal swab was immediately agitated into 0.9% saline solution. A drop of this was placed on the centre of a clean microscope slide and covered with a coverslip. The slide was initially carefully scanned at ×100 with a light microscope for motile trichomonads, pus cells, yeast cells and epithelial cells, and then at ×400 to confirm motility, flagella movement and morphological features of *T vaginalis*.

InPouch culture was carried out according to the manufacturer's instructions.[14] Pouches not registering the growth of trichomonads after the fifth day were declared negative.

Before specific PCR analysis, a check was carried out on the adequacy or quality of vaginal, oral and rectal swab sampling. Human β-2 microglobulin gene amplification[15] was carried out on the vaginal and oral swab eluates. Samples in which β-2 microglobulin gene sequences could not be amplified after a neat or a 1:10 dilution were considered to have PCR inhibitors or to have been poorly collected. The quality of rectal swab sampling was determined by the amplification of *Escherichia coli* sequences.[16] *E coli*, a commensal of the gut, is universally present in faecal material.

Two PCR assays amplifying different sequences of *T vaginalis* were used for the detection of *T vaginalis*. All vaginal swabs were screened with the primer set described by Kengne *et al*.[17] Confirmation of *T vaginalis*-positive results was carried out using the inner primer set designed by Shaio *et al*.[18] Overall cycling and testing conditions are described by Crucitti *et al*.[19] A specimen was considered positive for *T vaginalis* if both PCR assays amplified the *T vaginal*is target. The vaginal swab eluates on which *T vaginalis* PCR was performed were also tested for *P hominis* and *T tenax* using specific rRNA PCR primer sets.[11 20] PCRs for *P hominis* and *T tenax* on the rectal and oral swabs, respectively, were carried out using the same primers and cycling conditions as used on the vaginal swab eluates. Table 1 shows the trichomonad primer sequences used in the study.

T vaginalis wet-mount microscopy and InPouch culture were carried out at the Komfo Anokye Teaching Hospital, Kumasi, Ghana, and the swabs for PCR testing were stored at −20℃ until shipment on dry ice to the London School of Hygiene and Tropical Medicine (LSHTM), London, UK, and the Institute for Tropical Medicine, Antwerp, Belgium. PCR testing of vaginal swabs for *T vaginalis*, *P hominis* and *T tenax* were performed in London, whereas PCR of rectal swabs for *P hominis* and oral swabs for *T tenax* were performed in Antwerp. Ethical approval for the study was obtained from the Committeee on Human Research Ethics and Publication of the School of Medical Sciences, Kumasi, Ghana, and the Research Ethics Committee, LSHTM.

RESULTS

Samples from 3807 women were tested for *T vaginalis* infection by latex agglutination, of which 633 (206 positive, 427 negative) were tested for *T vaginalis* by other tests as per protocol. A total of 173 of the 206 women tested positive by the expanded gold standard wet-mount microscopy or InPouch culture and were enrolled as cases, and all the 427 latex negatives tested negative and were analysed as controls. Participants with discordant latex and expanded gold standard test results were not analysed in the case–control study. The prevalence of *T vaginalis* was 4.9% by the morphologically based expanded gold standard of culture or wet preparation.

The participants were aged 15–44 years, with a median age of 26 years. On univariate and age-adjusted analysis, *T vaginalis* infection was significantly associated with young age (<20 years; odds ratio (OR) 3.73, 95% CI 2.0 to 7.0, $p = 0.0001$), being single (OR 3.18, 95% CI 2.0 to 8.4, $p = 0.03$) and being of neither Akan nor northern ethnicity (OR 2.89, 95% CI 1.4 to 5.6, $p = 0.005$). Poor anogenital hygiene practices and the sexual practices studied were not associated with infection.

Of the 173 samples from which *T vaginalis* was identified by wet mount or culture, 93% (161) were evaluable by PCR. β-2 microglobulin gene sequences could not be detected in the other 12 samples, which were thus declared to have PCR assay inhibitors or to have been poorly collected. A total of 94% (152) of the 161 samples were also positive by *T vaginalis* PCR. β-2 microglobulin and *E coli* sequences were detected in all oral swab and rectal swab eluates, respectively.

P hominis and *T tenax* were not detected by PCR in any of the vaginal swab eluates. This included the nine samples for which *T vaginalis* PCR was negative, although trichomonads were detected by wet preparation or culture. PCR for *P hominis* and *T tenax* were negative in all the 600 rectal and oral swab samples, respectively.

DISCUSSION

Debate on the involvement of rectal trichomonads in the aetiology of vaginal trichomoniasis seemed to have been resolved in the 1930s and 1940s, with studies on cross infection and laboratory studies during which trichomonads inoculated outside their traditional habitats did not survive. These studies reported that trichomonads were site specific and could not survive outside their traditional habitats.[1–4 8 9] However, recent reports on the presence of trichomonads in non-traditional sites, with the use of molecular diagnostic methods, indicate otherwise.[21–24]

Table 1 Primer sequences used for *Trichomonas* spp

Organism	Primer pairs	Reference
Trichomonas vaginalis	5′-ATTGTCGAACATTGGTCTTACCCTC-3′ 5′-TCTGTGCCGTCTTCAAGTATGC-3′	Kengne *et al*[17]
	5′-AACATCCCCAACATCTT-3′ 5′-CCATTCTTTTAGACCCTT-3′	Shaio *et al*[18]
Pentatrichomonas hominis	5′-TGTAAACGATGCCGACAGAG-3′ 5′-CAACACTGAAGCCAATGCGAG-3′	Crucitti *et al*[11]
Trichomonas tenax	5′-AGTTCCATCGATGCCATTC-3′ 5′-GCATCTAAGGACTTAGACG-3′	Press *et al*[21]

The difficulty in morphological differentiation of trichomonads has also been highlighted in veterinary medical practice. Romatowski[25] initially reported isolating *P hominis* from the gut of kittens. Later, Levy *et al*[26] reported that these flagellates had been misdiagnosed, and that gene sequencing had identified the isolates as *Tritrichomonas foetus*. The practice of culling bulls with positive preputial cultures for the sexually transmitted pathogen *Tritrichomonas foetus* for economic reasons (causes infertility in herds of virgin bulls) has been questioned. Until recently, the diagnosis of *T foetus* preputial infection had been by microscopic examination and culture of preputial scrapings or washings. Using a combination of culture and PCR assays using *T foetus*-specific primers and pan-trichomonad primers, workers have observed the existence of easily misidentified non-*T foetus* trichomonads (most of which originate from the gut) in the bovine prepuce. They recommend that culture of preputial scrapings or washings should be augmented with specific PCR assays in confirming infection.[27 28]

In the population of pregnant women studied, we found no evidence for the involvement of rectal or oral trichomonads in cases of vaginal trichomoniasis using NAAT. A total of 94% of cases for which *T vaginalis* was detected by traditional methods (wet preparation or culture) were confirmed by *T vaginalis*-specific PCR assays. *T vaginalis* could not be detected by PCR in nine samples in which morphological identification was positive. All nine samples were concordant negative on the two *T vaginalis* PCR assays. Neither *P hominis* nor *T tenax* were found in these nine samples by specific PCR assays. This result could mean false-positive morphological *T vaginalis* diagnosis, poor specimen preparation or genetic variation of *T vaginalis*. Genetic variation of *T vaginalis* is likely to be the case, as the samples were both wet preparation and culture positive, and did not have any PCR inhibitors—that is, they were positive for β-2 microglobulin. Furthermore, Crucitti *et al*[19] and Pillay *et al*[29] have recently shown that the sensitivity of different PCR assays for the detection of *T vaginalis* infection varies.

P hominis, a gut commensal, has normally been reported in children from areas with poor hygiene. It is not surprising that none was detected in the study population because they were adult women. Similarly, *P hominis* was detected in stool samples from 112 adults in Belgium who had spent various lengths of time in the tropics.[11] *T tenax* has been reported especially in diseased mouths, which was not evident in the population of women studied.

The study also looked at various behavioural factors (sexual practices, anogenital hygiene and practices) that could seed *P hominis* and *T tenax* into the vagina. None of these was associated with *T vaginalis* infection.

Briefly, with the use of a combination of traditional and molecular diagnostic tests for trichomonads, no evidence for the involvement of trichomonads other than *T vaginalis* in the aetiology of vaginal trichomoniasis was found in this population with low prevalence of *T vaginalis*.

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